

### **REMARKS**

The Office Action mailed June 14, 2004 has been carefully reviewed and the foregoing amendments are made in response thereto. In view of the amendments and the following remarks, Applicants respectfully request reconsideration and reexamination of this application.

#### ***Objection to the Claims***

In paragraph 2 the Examiner objects to claims 20, 25, 29 and 33. Applicants have amended these claims to include "the" before "fragments" as suggested by the Examiner.

In paragraph 3-6 the Examiner objects to claims 20, 25 and 29 because of the terms sequence and sequences. As suggested by the Examiner, Applicant has replaced the terms sequence and sequences with the terms base or bases. Claim 33 has been similarly amended for consistency.

#### ***Rejection under 35 USC § 112, second paragraph***

In paragraphs 9, claims 20, 25, 29, and 33 have been rejected because the term "the fragments" is used in both the amplifying step and the ligating step. These claims have been amended herein to distinguish the fragments resulting from digestion with the fragments generated by adaptor ligation. The fragments resulting from digestion are referred to as "the fragments" and the fragments generated by adaptor ligation are referred to as the "adaptor-ligated fragments".

In paragraph 10, claims 20, 25, 29 and 33 have been rejected because the term "the fragments" is used in the providing step. Applicant has amended the claims to require that the fragments in the providing step are the fragments that are predicted to be amplified fragments resulting from the amplifying step.

In paragraph 11, claim 24 has been rejected because it is unclear as to whether the term "the fragments" refers to the fragments from the ligating step of the fragments generated from the amplifying step. The claim has been amended to require that the fragments are the fragments predicted to be amplified in the amplified fragments.

In paragraph 12, claims 35-39 have been rejected because of the use of the term "the fragments". The claims have been amended to clarify that it is the adaptor-ligated fragments

containing both the first and the second adaptor that comprise the specified percentage of the nucleic acid sample.

In response to the rejections of claims 43 and 46 in paragraphs 13 and 14 Applications have amended claims 43, 46 and 47. Claim 43 has been amended to clarify that it is ligation of the second adaptor that is blocked by the absence of a phosphate at the 5' end of one strand of the adaptor. Claim 46 has been amended to clarify that ligation of the second adaptor is blocked at the 5' end of one strand of the second adaptor and that ligation of the first adaptor is blocked at the 3' end of one strand of the first adaptor. Claim 47 has been amended to clarify that the second adaptor is blocked at the 5' end of both strands of the second adaptor and the first adaptor is blocked at the 3' end of both strands of the first adaptor.

***Rejections under 35 USC § 103(a)***

In paragraph 16, the Examiner rejected claims 20, 22, 24-27, 33, 40, 43 and 46 under 35 USC § 103(a) over McCasky Feazel *et al.* (U.S. Patent No. 6,100,030) in view of Pedersen (US 2003/0113737). Applicants traverse this rejection.

On page 6 of the office action, the Examiner asserts that McCasky Feazel *et al.* disclose enrichment of the EcoRI/MseI fragments relative to the MseI/MseI or EcoRI/EcoRI fragments. The Examiner points to figure 1 of McCasky Feazel for support. Applicants respectfully disagree. McCasky Feazel does not teach any method for preferentially amplifying the fragments cut by both enzymes relative to the fragments cut only by one or the other enzyme.

As the Examiner has indicated, the products of the digestion with Mse I and Eco RI would be MseI/MseI, EcoRI/EcoRI and MseI/EcoRI fragments. The Eco RI adaptor ligates to sites cut with EcoRI and the MseI adaptor ligates to sites cut with MseI, resulting in three types of adaptor ligated fragments: MseI-Adpt/MseI-Adpt, EcoRI-Adpt /EcoRI-Adpt and MseI-Adpt /EcoRI-Adpt. The MseI-Adpt/MseI-Adpt and EcoRI-Adpt /EcoRI-Adpt adaptor ligated fragments are substrates for amplification in the subsequent amplification reaction and will be amplified with the same efficiency as the MseI-Adpt /EcoRI-Adpt adaptor-ligated fragments.

MseI has a recognition site that is 4 base pairs and EcoRI has a 6 base pair recognition site, as a result the frequency of MseI sites is approximately once every 256 base pairs and EcoRI sites approximately once every 4096 base pairs. As a result the predominant product will be the MseI-Adpt/MseI-Adpt adaptor ligated fragments and those will be amplified. McCasky

Feazel et al. fails to teach any method by which the amplification of the MseI/MseI or EcoRI/EcoRI fragments would be inhibited in the amplification reaction. Figure 1 of McCasky Feazel is simply a schematic that shows one of the three types of fragments possible in the reaction-the other two fragment types aren't shown but there is no teaching of a method to amplify the EcoRI/MseI fragments over the EcoRI/EcoRI and MseI/MseI fragments.

Pederson fails to remedy the deficiencies of McCasky Feazel et al. Paragraph [0268] of Pederson teaches that "identifying linker nucleotides" can be blocked from ligation at the 5' or 3' ends of either strand or both strands. There is no teaching or suggestion of blocking a first adaptor from ligation at the 3' end and a second adapter from ligation at the 5' end as required by the claims. The blocking of ligation of one adaptor at the 5' end and the other adaptor at the 3' end in the present invention allows for selective amplification of only those fragments that have both adaptors ligated to the ends of the fragment, because only those fragments have a contiguous template strand containing both the first adaptor primer site and the second adaptor primer site. Neither Pederson nor McCasky Feazel teaches or suggests this limitation and it would not be prima facie obvious to one having ordinary skill in the art to have performed the methods recited in the claims in view of McCasky Feazel *et al.* and Pederson. Applicants respectfully request withdrawal of the rejection.

In paragraph 17 claims 29-31 are rejected under 35 USC § 103(a) over McCasky Feazel et al. in view of Pederson and in view of Guire *et al.* (U.S. Patent No. 6,514,768). As discussed above McCasky Feazel et al. in view of Pederson fails to teach a method of selectively amplifying those fragments that are cut on one end by a first enzyme and on the other end by a second enzyme. Guire et al. fails to remedy the deficiencies of McCasky Feazel et al. and Pederson. Applicants respectfully request withdrawal of the rejection.

In paragraph 18 claims 35-39 are rejected under 35 USC § 103(a) over McCasky Feazel *et al.* in view of Pederson and further in view of Short *et al.* (U.S. Patent No. 6,238,884). As indicated above, McCasky Feazel et al. and Pederson fail to teach a method to preferentially amplify those fragments that are ligated to both a first and second adaptor relative to those fragments ligated to only the first adaptor or to only the second adaptor as required by amended claims 20, 25, 29 and 33. Short *et al.* also fail to teach a mechanism to preferentially amplify those fragments that are ligated to both a first adaptor and a second adaptor. Therefore, Short *et al.* fails to remedy the deficiencies of McCaskey Feazel *et al.* and Pederson. Applicants

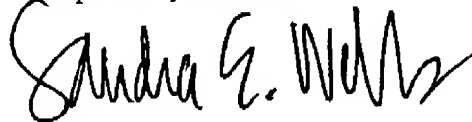
respectfully request withdrawal of the rejection.

**CONCLUSION**

In view of the foregoing amendments and remarks, Applicants believe all pending claims are now in condition for allowance and should be passed to issue. If the Examiner feels that a telephonic interview would in any way expedite the prosecution and allowance of this application, please do not hesitate to call the undersigned at (408) 731-5768. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account 01-0431.

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Respectfully submitted,



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